

What is claimed is:

1. An in vitro method of activating protein kinase B comprising
  - (a) obtaining from an insulin-responsive cell a membrane fraction and a cytoplasmic fraction, which comprises a protein kinase B,
  - (b) combining the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride, wherein
  - (c) the protein kinase B is activated by virtue of having a threonine residue and a serine residue phosphorylated, such that
  - (d) the activated protein kinase B is capable of phosphorylating a GSK3.
2. The method of claim 1 wherein the insulin-responsive cell is treated with insulin.
3. The method of claim 2 wherein the membrane fraction is a plasma membrane fraction.
4. The method of claim 1 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.
5. The method of claim 1 further comprising the step of combining PIP3 or PI(3,4)P2 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.
6. The method of claim 5 further comprising the step of combining PIP3 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.
7. The method of claim 1 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.
8. The method of claim 1 wherein the insulin-responsive cell is an adipocyte.
9. An in vitro method of activating protein kinase B comprising
  - (a) obtaining from an insulin-responsive cell a plasma membrane fraction and a cytoplasmic fraction, which comprises a protein kinase B,
  - (b) treating said plasma membrane fraction with a solution comprising at least 145 mM chloride, thereby obtaining a salt-extracted plasma membrane fraction and an aqueous fraction,

(c) desalting the aqueous fraction thereby producing a desalted aqueous fraction comprising less than 145 mM chloride,

(d) combining the salt-extracted plasma membrane fraction, the cytoplasmic fraction, the desalted aqueous fraction, ATP, and a phosphatidylinositol phosphate molecule in a buffer comprising less than 145 mM chloride, wherein

(e) the protein kinase B is activated by virtue of having a threonine residue and a serine residue phosphorylated, such that

(d) the activated protein kinase B is capable of phosphorylating a GSK3.

10. The method of claim 9 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.

11. The method of claim 9 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.

12. The method of claim 9 wherein the insulin-responsive cell is an adipocyte.

13. The method of claim 9 wherein the insulin-responsive cell is treated with insulin.

14. The method of claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3 or PI(3,4)P2.

15. The method of claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3.

16. An in vitro method of phosphorylating a serine of protein kinase B comprising

(a) obtaining from an insulin-responsive cell a membrane fraction and a cytoplasmic fraction, which comprises a protein kinase B,

(b) combining the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride, wherein

(c) the protein kinase B is phosphorylated at a serine residue.

17. The method of claim 16 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1.

18. An in vitro method of identifying an agent that modulates insulin activity comprising

(a) obtaining from an insulin-responsive cell (i) a membrane fraction, which comprises a phosphatidylinositol(3,4,5)P<sub>3</sub>-dependent protein kinase-2 ("PDK2") activity

and an insulin receptor, and (ii) a cytoplasmic fraction, which comprises a protein kinase B and a phosphatidylinositol(3,4,5)P<sub>3</sub>-dependent protein kinase-1 ("PDK1") activity,

(b) combining the membrane fraction, the cytoplasmic fraction and ATP with the agent in a buffer comprising less than 145 mM chloride, and

(c) assessing the phosphorylation status of the protein kinase B.

19. The method of claim 18 wherein the insulin-responsive cell is treated with insulin.

20. The method of claim 18 wherein the membrane fraction is a plasma membrane fraction.

21. The method of claim 18 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.

22. The method of claim 1 further comprising the step of combining PIP3 or PI(3,4)P2 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.

23. The method of claim 24 further comprising the step of combining PIP3 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.

24. The method of claim 18 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.

25. The method of claim 18 wherein the insulin-responsive cell is an adipocyte.

26. The method of claim 18 wherein the phosphorylation status of protein kinase B is assessed by immunoblot analysis using phospho-Akt antibodies.

27. A composition comprising a prepared membrane fraction obtained from a cell, wherein said prepared membrane fraction comprises an enzyme having PDK2 activity, wherein said PDK2 activity includes the phosphorylation of a serine residue of protein kinase B.

28. The composition of claim 27 wherein the cell is an insulin-responsive cell selected from the group consisting of islet cell, muscle cell, liver cell and adipocyte.

29. The composition of claim 27 wherein the cell is an adipocyte.